HOW ARE MICE MAINTAINED FREE OF PATHOGENS?

Division of Comparative Medicine

1. Purpose

Comparative Medicine's centralized service of **specific pathogen-free** (SPF), **viral antibody-free** (VAF) animal procurement, husbandry, health surveillance, and quality control ensures research **data integrity** and reproducibility. The difficulty of **infectious agents**, apart from the obvious potential for clinical morbidity and mortality of unique mouse models, is that they **interfere with the normative responses of even asymptomatic mice**, including altering numerous cell biological responses *in vitro*, resulting in data variation and the misinterpretation of results.

2. Procedures

Efforts are directed toward 1) **excluding** potential pathogens, 2) **deriving and maintaining mouse colonies in a barrier setting**, 3) **defining the microbial status** of mouse colonies, 4) **reducing risks** of infectious agent exposure, 5) **rederivation** of infected colonies, and 5) **increasing awareness** of microbial effects on data interpretation.

3. Quarantine

Shipping boxes containing SPF/VAF rodents from **approved vendors** (i.e., The Jackson Laboratories, Inc., Harlan Sprague Dawley, Inc., Charles River Laboratories, Inc., and Taconic, Inc.) are inspected for signs of damage, shipping accuracy, sprayed lightly with Clidox, allowed to dry, and then taken to an assigned housing room. Mice from unapproved sources (i.e., universities, research institutes, and unapproved vendors) are quarantined for five weeks accessible to only Comparative Medicine staff, then their health status and eligibility for release from quarantine determined by the assigned veterinarian as described below. While in quarantine mice are housed in individually ventilated cage/rack system and provided Ivermectin topical treatments and Febendazole medicated chow.

During quarantine, mice are examined for evidence of endo- and ectoparasites. Fresh feces are mixed in a super-saturated salt solution and the buoyant material examined microscopically for helminth ova and protozoal oocysts. Cellophane tape exposed to the perineum is examined for pinworm ova. Immunologically competent mice are tested for serological evidence of exposure to microbes on the division's **infectious agent exclusion list**. Representative mice are bled for either a clinical serological profile for evidence of exposure to Parainfluenza virus Type 1 (Sendai), Coronavirus (MHV), Mycoplasma pulmonis, Parvovirus (MVM and MPV), Poliovirus (TMEV-GDVII), and Rotavirus (EDIM), or a comprehensive serological profile which includes, in addition to the agents listed above, Paramyxovirus (PVM) Reovirus Type 3, Lymphocytic Choriomeningitis Virus, Mouse Adenovirus types 1 & 2, Poxvirus (Ectromelia), Papovavirus (Poly) and Calicivirus (MNV). Additional health evaluations (e.g., polymerase chain reaction (PCR) screening of fecal pellets for Helicobacter sp., bacterial culture, isolation, and identification of enteric or respiratory potential pathogens) are requested at the discretion of the veterinarians upon consultation with the research staff. A service of cesarean rederivation, embryo transfer, and fostering of valuable mice is provided.

4. Animal Health Monitoring and Surveillance

Sentinel mice are evaluated at least every six months, during the months of May and November, and whenever required. SPF/VAF mice are ordered from approved vendors, and assigned to a rack-side in a housing room. Each sentinel is identified by a unique code that indicates facility, room, rack location, and rack-side. An assumed 50% infectivity rate and 95% confidence interval are used to determine the number of sentinels to place, so that approximately 1 sentinel mouse is placed for every 35 microisolators. Sentinels are exposed to soiled bedding from occupied microisolators present on the same rack-side for a period of 10 weeks and euthanatized for evaluation.

After the 10 week exposure/incubation period, sentinels undergo direct evaluation of the hair, skin, pelage for evidence of ectoparasites, direct evaluation of the cecum and intestine for endoparasites, parasitology fecal examinations as described above, a gross necropsy with dissection and collection of lesions for histological description and microbiological evaluation when appropriate, and are bled and tested for a serological panel for evidence of exposure to Parainfluenza virus Type 1 (Sendai), Coronavirus (MHV), Mycoplasma pulmonis, Parvovirus (MVM and MPV), Poliovirus (TMEV-GDVII), and Rotavirus (EDIM) . The serological testing described above is run on all sentinel samples semiannually. Additional health evaluations are conducted at the discretion of the veterinarians upon consultation with the research staff.

Sentinel mice housed at the Stabile Research Building (SRB) barrier facility are tested for the following additional agents using a comprehensive serological profile: Paramyxovirus (PVM), Reovirus Type 3, Lymphocytic Choriomeningitis Virus (LCM), Mouse Adenovirus (MAD 1&2), Poxvirus (Ectromelia), Papovavirus (Polyoma), and Calicivirus (MNV). Helicobacter is included on the infectious agent exclusion list for this facility, and sentinel animals are tested semiannually for this agent by PCR. A database of all animal health evaluations, findings, interpretations, treatments, and management decisions is maintained by Comparative Medicine.

Biologics, cultured cells, or transplantable tumors to be administered to mice should be sterile preparations, pure characterized cultures, or PCR tested, or otherwise characterized as free of microbes that can infect mice prior to their use in any facility. A PCR-based testing panel is available to evaluate biologics for the presence of the agents on the infectious agent exclusion list with the exception that no tests to detect Cilia-associated Respiratory Bacillus, Clostridium piliforme (Tyzzer's), or Encephalitozoon cuniculi are conducted.

5. Pathogen Exclusion Standard Procedures

Each animal facility is a distinct and secure area, nonpublic and separate from research, teaching, or clinical areas. Access to each facility and to many of the individual housing, use, service, and storage areas is controlled by key, or swipe card and security codes. Authorized personnel certified by the IACUC don disposable gloves, gowns, and shoe covers before entering animal areas. Mouse production colony housing areas are as separate from experimental mouse housing areas as is feasible. Separate experimental mouse-housing areas are established for immunocompromised colonies, for colonies administered carcinogens, or recombinant DNA, or biohazardous agents of NIH Risk Groups II or I. To optimize the mouse primary enclosure environment, to maintain mouse health security, and to minimize personnel exposures to mouse-expressed human allergens, mice are housed in individually ventilated cage/rack system or in static microisolators. Sterile cages are opened and changed within mobile dual-sided animal transfer stations that provide HEPA downward laminar airflow and a disinfected work surface whenever possible. Mice housed in sterile caging are provided sterile drinking water and fed irradiated chow, unless mice are on a protocol-specific diet.

6. Limited Movement of Animals

Transportation of animals, including intra-institutional transportation, occurs only when essential since any transit time introduces risks of exposure to environmental extremes, crowding, infectious agents, and possible zoonoses, which can affect animal and public welfare, and the consistency of results. Movement of animals between animal housing rooms, or between separate facilities is discouraged, and permitted only when requested in writing using a "*Request to Relocate Research Animals*" form or a "*Request to Reassign Locally Produced Mice to a Research Protocol*" form, and approved by Comparative Medicine. Shipment of animals to or from other institutions must be requested in writing using a "*Request to Ship Mice to Another Institution*" form, or a "*Request to Ship Rats to Another Institution*" form, or a "*Request to Receive Animals From Another Institution*" form, approved by Comparative Medicine, and arranged and accomplished by Comparative Medicine, in compliance with the AWR and the International Air Transport Association Live Animal Regulations (2009).

7. Effective Decontamination

All caging and equipment is mechanically sanitized in an automated cage/rack washer. A water temperature minimum of 160º F is monitored and logged daily. Each week tri-temp-tapes are run and logged. Each quarter caging and equipment are monitored for efficacy of sanitation by using a Lum-T Luminometer® to detect the presence of adenosine triphosphate (ATP). Results of monitoring are maintained in a database, and corrective actions, if required, are documented. An Amsco Model 6000 tunnel washer and Model 4600 rack washer are installed in the College of Medicine facility. Two Amsco Model 6000 tunnel washers and an Amsco Model 9500 rack washer are installed at the Stabile Research Building; an Amsco 9500 rack washer is installed in the James A. Haley VA Hospital facility; a Cesco tunnel washer and an Amsco Model 9700 cage and rack washer are installed in the All Children's Hospital facility; an Amsco Model 3700 cage and bottle washer is installed in the College of Public Health; an Amsco Model 9500 cage and rack washer is installed at the Psychology facility; and an Amsco Model 4600 cage and rack washer and an Amsco Model 6000 tunnel washer is installed at the Bird Alzheimer's Institute facility. Autoclaves are assessed and results logged using sterilizer monitoring strips in each load, and using Verify indicators containing spores of *Bacillus stearothermophilus* and *Bacillus subtilis var. niger*, at least weekly. Decon-Spore 200 Plus is used in automated floor machines for sanitizing seamless corridor floors, and either Sporicidin or Clidox in spray bottles to decontaminate countertops and equipment. Clidox is used in an aerosol fogger during animal room decontamination procedures, the efficacy of which is determined by using a Lum-T luminometer, as above. Pest control is assisted by the surface application of pyrethrin insecticides around the exterior perimeter of facilities, and at the baseboard of the interior corridors and service areas as requested by the facility manager.